TABLE 58.2
Enzyme Separation

	Year	Author	Enzyme	Concentration	Ideal Dilution	Duration Time
1	1941	Medwar PB [28]	Trypsin	0.5%	Tyrode's solution	Depends on skin thickness
	1962	Klein M [21]	Trypsin	0.5%	Tyrode's	30 min(back skin), 25 min(ear skin)
	1966	Einbinder JM [8]	Trypsin	0.025%	Tris buffer (0.05M)	90 min~4 hr (37°C)
	1958	Fan J [11]	Purified trypsin; crystaling trypsin	0.5% 0.1%	Isotonic solution (NaCl 0.042%, KCl 0.42%, $CaCl_2$ 4.2%, $NaHCO_3$ 0.15%, glucose 0.1%)	45 min (40°C)
	1985	Takahashi H [35]	Trypsin	0.25%	CMF-BSS (Ca^{++} and Mg^{++} free Hank's balanced salt solution)	12~13 hr (4°C)
2	1952	Becker SW [2]	Pancreatin	0.25%~0.5%	Ringer-sodium bicarbonate solution	10~15 min (38°C)
	1958	Fan J [11]	Pancreatin	0.5%	Isotonic solution (NaCl 0.042%, KCl 0.42%, $CaCl_2$ 4.2%, $NaHCO_3$ 0.15%, glucose 0.1%)	45 min (40°C)
	1966	Einbinder JM [8]	Pancreatic elastase	0.05%	Tris buffer (0.05M)	90 min~4hr (37°C)
3	1962	Klein M [21]	Elastase	0.012%	Tyrode's	20~25 min(back skin),10~15 min(ear skin)
	1966	Einbinder JM [8]	Fungal elastase	0.1%	Tris buffer (0.05M)	90 min~4 hr (37°C)
4	1966	Einbinder JM [8]	Keratinase	0.1%	Tris buffer (0.05M)	30 min (37°C)
5	1966	Einbinder JM [8]	Collagenase	0.1%	Tris buffer (0.05M)	30 min (37°C)
6	1966	Einbinder JM [8]	Pronase	0.1%	Tris buffer (0.05M)	30 min (37°C)
7	1989	Walzer C [40]	Thermolysin	250 & 500 ug/ml	Mg^{++}-free PBS	1 hr (4°C)
8	1983	Kitano Y [20]	Dispase	500 & 1000 U/ml	PBS, Eagle MEM, or Eagle MEM supplemented with 20% fetal bovine serum	24 hr (4°C)
	1985	Takahashi H [35]	Dispase	1000 U/mL	M-199 containing 10% FCS	24 hr (4°C)
	1995	Ohata Y [31]	Dispase	1000 U/mL	Dulbecco's modified Eagle medium	45 min (37°C)

elastase, and keratinase (Table 58.3). The endpoint of digestion was similar. Optimal enzyme concentration, pH, and time varied. Pronase was extremely active, although its proteolytic and elastolytic activity were not significantly different from the others. Separation at the EDJ was followed by acantholysis and subsequently loss of dermal appendages and fibrous elements. Mature keratin structures or basement membrane structures did not appear altered.

58.3.2.4 Dispase

Dispase is a bacterial neutral protease obtained from the *Bacillus polymyxa* culture filtrate, and its cytotoxicity is low. Kitano et al. isolated epidermal sheets *in vivo* without dissociating cells [20]. After 24 hours human skin treatment with 500 and 1000 U/mL dispase, epidermal sheets were easily peeled from the dermis, and its undersurface retained rete ridges. Electron microscopic observation showed the basal surface composed of cells with numerous slender villi and cytoplasmic